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**LDN®**

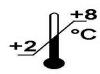
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**Instructions for use**  
**HistaSure™ ELISA Fast Track**



**REF**

**FC E-3600**



## **1. Intended use and principle of the test**

The **HistaSure™ ELISA Fast Track** is intended for the rapid semi-quantitative or quantitative determination of histamine in different scombrotoxin fish types such as tuna, mahi mahi, sardines and for the determination of histamine in fishmeal.

The assay kit provides materials for the determination of derivatized histamine in food extracts. The derivatization is part of the preparation of the samples. By use of the acylation reagent, histamine is quantitatively derivatized into N-acylhistamine. The competitive Histamine ELISA kit uses the microtiter plate format. Histamine is bound to the solid phase of the microtiter plate. Acylated histamine and solid phase bound histamine compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum-peroxidase complexes are removed by washing. The substrate TMB/peroxidase reaction is monitored at 450 nm. The amount of antibody bound to the solid phase histamine is inversely proportional to the histamine concentration of the sample.

## **2. Introduction**

Histamine testing in fresh fish is a possible control strategy that can be used by seafood processors in their HACCP program to address the hazard of scombrotoxin formation. Histamine is a product of decomposition of histidine caused by the growth of certain bacteria in seafood. The amount of the amine that forms is a function of bacterial species, the temperature and time of exposure, and may exceed 1,000 ppm (mg/kg). Fish containing high levels of histamine has been associated with many examples of poisoning commonly referred to as "scombrotoxin poisoning," a major health problem for consumers. Scombrotoxic fish usually contains levels of histamine in excess of 200 ppm but such fish may be randomly dispersed within a lot. For large fish, histamine is found at variable levels even within individual fish. Quality control measures designed to minimize the occurrence of scombrotoxic fish require the determination of histamine levels in the range of approximately 10 to 200 ppm. Good quality fish contain less than 10 ppm histamine, a level of 30 ppm indicates significant deterioration, and 50 ppm is considered to be evidence of definite decomposition. The defect action level (DAL), the level at which regulatory actions are taken for histamine is 50 ppm (P. L. Rogers, W. F. Staruszkiewicz, Journal of Aquatic Food Product Technology, Vol. 9 (2) 2000 p. 5 - 17).

## **3. Procedural Cautions, Guidelines and Warnings**

- This kit is for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- In order to reduce exposure to potentially harmful substances, wear lab coats, disposable latex gloves and protective glasses where necessary.
- All kit reagents and specimens should be brought to room temperature (20 - 25 °C) and mixed gently but thoroughly before use.
- When the use of water is specified for dilution or reconstitution, use deionized, distilled, or ultra-pure water.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided. Wells are for single use only.
- Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time.
- Incubation times do influence the results. All wells should be handled in the same order and time sequences.
- To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
- Do not mix various lot numbers of kit components within a test and do not use reagents beyond expiry date as shown on the kit labels.
- Avoid contact with Stop Solution containing 0.25 M H<sub>2</sub>SO<sub>4</sub>. It may cause skin irritation and burns. In case of contact with eyes or skin, flush immediately with water.
- Some reagents contain sodium azide (NaN<sub>3</sub>) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN<sub>3</sub> may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets (MSDS). The Material Safety Data Sheet for this product is available directly on the website of the manufacturer or upon request.
- Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

#### 4. **Storage and stability**

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiration date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

#### 5. **Materials**

##### 5.1 **Contents of the kit**

<b>REF</b>	<b>Symbol</b>	<b>Reagent</b>	<b>Content</b>	<b>Colour Code</b>
<b>BA D-0035</b>	□□□ MB 48	<b>Master Block</b>	1 x 48 wells	ready for use
<b>BA E-0030</b>	WASH-CONC 50x	<b>Wash Buffer Concentrate</b>	1 x 20 ml light purple	concentrate
<b>BA E-0055</b>	SUBSTRATE	<b>Substrate</b>	1 x 12 ml black	ready for use, containing a solution of (TMB)
<b>BA E-0080</b>	STOP-SOLN	<b>Stop Solution</b>	1 x 12 ml light grey	ready for use
<b>FC E-3601</b>	C⇒0 PPM	<b>Control 0 ppm</b>	1 x 4 ml white	ready for use
<b>FC E-3602</b>	C⇒3 PPM	<b>Control 3 ppm</b>	1 x 4 ml light yellow	ready for use
<b>FC E-3603</b>	C⇒10 PPM	<b>Control 10 ppm</b>	1 x 4 ml orange	ready for use
<b>FC E-3604</b>	C⇒20 PPM	<b>Control 20 ppm</b>	1 x 4 ml light green	ready for use
<b>FC E-3605</b>	C⇒30 PPM	<b>Control 30 ppm</b>	1 x 4 ml light purple	ready for use
<b>FC E-3606</b>	C⇒50 PPM	<b>Control 50 ppm</b>	1 x 4 ml dark blue	ready for use
<b>FC E-3607</b>	C⇒100 PPM	<b>Control 100 ppm</b>	1 x 4 ml light grey	ready for use
<b>FC E-3608</b>	C⇒300 PPM	<b>Control 300 ppm</b>	1 x 4 ml black	ready for use
<b>FC E-3611</b>	ACYL-BUFF	<b>Acylation Buffer</b>	2 x 50 ml white	ready for use
<b>FC E-3612</b>	ACYL-REAG	<b>Acylation Reagent</b>	1 x 3 ml brown	ready for use, yellow coloured
<b>FC E-3631</b>	W HIS	<b>Histamine Microtiter Strips</b>	1 x 48 wells	6 strips, 8 wells each, break apart, precoated
<b>FC E-3640</b>	HIS AB CONJ	<b>Histamine Antiserum Conjugate</b>	1 x 6 ml red	ready for use, goat-anti Histamine IgG conjugated with peroxidase

##### 5.2 **Additional materials and equipment required but not provided with the kit**

- Precision pipette (50 µl)
- Pipette tips (50 µl)
- Manual repetitive pipette (e.g. the Brand HandyStep® S)
- Precision Dispenser Tips (5 ml, 25 ml; e.g. the PLASTIBRAND® PD-Tips)
- Grinder (mill) or house hold blender
- Graduated plastic cylinder (250 ml)
- Water (deionized, distilled, or ultra-pure)
- Scale (capable of weighing 5 – 50 grams, precision 0.1 gram)
- Funnel and filter paper (or alternatively a centrifuge)
- Timer
- Waterproof marker
- Absorbent material (paper towel)
- Microplate Vibration Shaker (shaking amplitude 2 mm; approx. 600 rpm, (e.g. PSU-2T Minishaker \*)
- ELISA reader capable of reading absorbance at 450 nm (required for semi-quantitative and quantitative determination)
- Washing device (plate washer or manually)

\* Available upon request!

## **6. Test procedure**

### **6.1 Preparation of reagents**

#### **Wash Buffer**

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml. Storage: up to 6 months at 2–8°C.

#### **Acylation Reagent**

The Acylation Reagent has a freezing point of 18.5 °C. To ensure that the Acylation Reagent is liquid when being used, it must be ensured that the Acylation Reagent has reached room temperature and forms a homogeneous, crystal-free solution before being used.

### **6.2 Sample preparation**

The following protocols for the sample preparations are based on the **AOAC Official Method 937.07**

Sampling should be performed according to national regulation.

#### **A. FRESH FISH • FROZEN FISH**

- Keep (fresh) fish frozen prior to analysis.
- Thaw samples under refrigeration or in cold water. Do **not** thaw the samples in a heated water bath. Discard draining.
- Once thawed, store the samples refrigerated (2 - 8 °C) prior to testing.

##### **WHOLE FISH:**

Clean, scale and eviscerate fish. In case of small fish 6 in. ( $\leq 15$  cm), use 5 – 10 fish. In case of large fish, from each of  $\geq 3$  fish cut 3 cross-sectional slices 1 in. (2.5 cm) thick, 1 slice from just back of pectoral fins, 1 slice halfway between first slice and vent, and 1 slice just back of vent. Remove bone. Blend combined samples until homogenous.

##### **FISH FILET:**

Use entire piece. Blend until homogenous.

#### **B. CANNED FISH and OTHER CANNED MARINE PRODUCTS**

Place entire content of the can (meat and liquid) in a blender and blend until homogenous.

#### **C. CANNED MARINE PRODUCTS PACKED in OIL, SAUCE, BRINE or BROTH**

Drain for 2 minutes on number 8 sieve or dab away the fluid with a paper towel. Place the meat in a blender and blend until homogenous.

#### **D. FISHMEAL**

Mix sample until homogenous.

### **6.3 Extraction**

- |                                                                                                                                                                                                                                                                                                                                                    |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"><li>• <b>Weigh 10 g</b> of prepared fish sample / fish meal, <b>add 240 ml water</b> (deionized, distilled, or ultra-pure) and <b>homogenize*</b>) for 1-2 minutes in a grinder or blender.<br/>*): Instead of homogenization <b>fish meal samples</b> are stirred for 10 minutes at room temperature.</li></ul> |
| <ul style="list-style-type: none"><li>• <b>Filter</b> the homogenate through folded filter paper (alternatively an aliquot of the homogenate can be centrifuged for 5 minutes at maximum speed). If a <b>lipid layer</b> forms remove it by suction!</li></ul>                                                                                     |
| <ul style="list-style-type: none"><li>• Use 50 µl of the <b>sample extract</b> for the acylation.</li></ul>                                                                                                                                                                                                                                        |

## 6.4 Histamine ELISA

For the subsequent steps (Acylation and ELISA) allow all reagents and samples to reach room temperature.

### A. SEMI-QUANTITATIVE DETERMINATION

For the semi-quantitative determination select the desired cut-off you need from the controls provided with the kit.

The kit controls have the following concentrations:

**control 3, 10, 20, 30, 50, 100 or 300 ppm.**

### B. QUANTITATIVE DETERMINATION

For the quantitative determination use the following controls provided with the kit:

**control 0, 3, 10, 30, 100 and 300 ppm.**

These 6 controls are used to establish the standard curve (please refer to section 7.)

#### 6.4.1 Acylation

<b>1.</b>	Pipette <b>50 µl</b> of <b>control(s)</b> and <b>sample extracts</b> into the respective wells of the <b>Master Block</b> .
<b>2.</b>	Add <b>1.5 ml</b> of <b>Acylation Buffer</b> in <b>1 (!)</b> pipetting step to all wells. <i>The use of a repetitive pipette together with a new Precision Dispenser Tip (25 ml, please refer to chapter 5.), is recommended.</i>
<b>3.</b>	Add <b>50 µl</b> of <b>Acylation Reagent</b> to all wells. ( <i>Colour change from yellow to pink!</i> ) <b>Continue without any delay with step 4!</b>  <i>The use of a repetitive pipette together with a new Precision Dispenser Tip (2.5 ml, please refer to chapter 5.), is recommended.</i>
<b>4.</b>	Incubate <b>5 min</b> at <b>RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm).  <b>Make sure that mixing is complete (slight pink colour).</b>
<b>5.</b>	Take <b>50 µl</b> for the ELISA

#### 6.4.2 Histamine ELISA

<b>1.</b>	Pipette <b>50 µl</b> of the <b>acylated control(s)</b> and <b>samples</b> into the wells of the <b>Histamine Microtiter Strips</b> .
<b>2.</b>	Pipette <b>100 µl</b> of the <b>Histamine Antiserum Conjugate</b> into all wells. <i>The use of a repetitive pipette together with a new Precision Dispenser Tip (5 ml, please refer to chapter 5.), is recommended.</i>
<b>3.</b>	Incubate <b>10 min</b> at <b>RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm).
<b>4.</b>	Discard or aspirate the contents of the wells. Wash the plate <b>3 x</b> by adding <b>300 µl</b> of <b>Wash Buffer</b> , <b>discarding</b> the content and <b>blotting dry each time</b> by tapping the inverted plate on absorbent material.
<b>5.</b>	Pipette <b>100 µl</b> of the <b>Substrate</b> into all wells. <i>The use of a repetitive pipette together with a new Precision Dispenser Tip (5 ml, please refer to chapter 5.), is recommended.</i>
<b>6.</b>	Incubate for <b>10 min</b> at <b>RT</b> (20 - 25 °C) on a <b>shaker</b> (approx. 600 rpm).  <b>Avoid exposure to direct sunlight!</b>
<b>7.</b>	Add <b>100 µl</b> of the <b>Stop Solution</b> to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution. <i>The use of a repetitive pipette together with a new Precision Dispenser Tip (5 ml, please refer to chapter 5.), is recommended.</i>
<b>8.</b>	<b>Read</b> the absorbencies of the solutions in the wells within 10 minutes using a <b>microplate reader</b> set to <b>450 nm</b> .

## **7. Calculation of results**

### **A. Semi-quantitative results:**

If the absorbance of the sample is **higher** than that of the selected **Cut-Off Control**, the sample has **passed**.

If the absorbance of the sample is **lower** than that of the selected **Cut-Off Control**, the sample has **failed**.

### **B. Quantitative results:**

The absorbance readings of the 6 controls (0, 3, 10, 30, 100 and 300 ppm) are used to establish a standard curve.

Plot the absorbance readings of the controls (y-axis, linear) against the corresponding control concentrations (x-axis, log) using a concentration of 0.001 ppm for the 0-control (*this alignment is mandatory because of the logarithmic presentation of the data*). For the curve fitting a non-linear regression has to be applied.

The concentrations of the samples can be read **directly** from this standard curve.

 *If a sample is off-curve it has to be diluted with water 1:10 and re-assayed. The result obtained has to be multiplied by the dilution factor of 10.*

## **8. Warranty**

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages occurring during transit.

## **9. Application lists for different kind of fish samples**

All fish samples tested so far are suitable for the **HistaSure™ ELISA Fast Track**. The lists below depict some major applications in different matrices.

### **9.1 Species validated through AOAC Certification**

<b>Fish Species</b>	<b>Presentation</b>
Tuna	- canned chunk light - fresh/frozen yellow fin
Mahi Mahi	- fresh/frozen
Sardines	- canned in oil
Fishmeal	

### **9.2 Species approved through in-house testing**

<b>Fish Species</b>	<b>Presentation</b>
Mackerel	- smoked
Anchovy	- fresh - brined - in sauce
Shad	- dry salted - fermented
Herring	- smoked
Salmon	- smoked
Bonito	- lakerda

## 10. Assay characteristics

**AOAC performance tested method for fresh/frozen yellowfin tuna, canned tuna-chunk light in water, frozen mahi mahi, canned sardines in oil and fish meal.**

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%) Histamine
	Histamine	100
	L-Tryptophan	nd
	Tryptamine	nd
	3-Methylhistamine	0.44
	L-Histidine	nd
	L-Tyrosine	nd
	L-Phenylalanine	nd
	Tyramine	0.69
	Cadaverine	0.40
	Spermine	nd
	Putrescine	nd
	Trimethylamine	nd

nd = not detectable

Accuracy and Precision					
Recovery			Intra CV		
Sample	Fortification range (ppm)	Mean recovery (%)	Recovery range (%)	Mean CV (%) (n=7)	Range CV (%)
Fresh/Frozen Tuna	5.25 – 218.6	91.8	85.2 – 99.6	7.58	4.13 – 10.8
Canned Tuna	5.49 – 267.3	99.8	94.7 – 106.5	8.74	3.17 – 13.9
Frozen Mahi Mahi	6.38 – 199.0	87.3	79.1 – 103.1	6.19	2.97 – 9.56
Canned Sardines	5.27 – 249.9	87.6	76.8 – 99.7	5.65	1.80 – 9.22
Fish meal	9.4 – 244.2	86.0	79.0 – 93.9	4.89	2.09 – 7.92

	Mean (ppm)	n	Inter CV %
Lot to Lot	25.8	3 lots	4.16

Detection limits	
LOD (Limit of Detection) 0.44 ppm	LOQ (Limit of Quantification) 1.31 ppm

Method Comparison	
LDN HistaSure Elisa vs AOAC 977.13 fluorometric method: Fresh/Frozen Tuna, Canned Tuna, Frozen Mahi Mahi and Canned Sardines	

Scatter plot showing the relationship between HistaSure Elisa histamine (ppm) on the y-axis and AOAC 977.13 histamine (ppm) on the x-axis. The x-axis ranges from 0 to 300, and the y-axis ranges from 0 to 300. A linear regression line is plotted with the equation  $y = 0.90x + 2.81$  and  $r^2 = 0.95$ . The data points show a strong positive correlation, with most points falling along the line.

AOAC 977.13 histamine (ppm)	HistaSure Elisa histamine (ppm)
10	10
20	20
30	30
40	40
50	50
60	60
70	70
80	80
90	90
100	100
110	110
120	120
130	130
140	140
150	150
160	160
170	170
180	180
190	190
200	130
210	250
220	260
230	200
240	220

**Symbols:**

	Contains sufficient for <n> tests		Manufacturer		Storage temperature
	Catalogue number		Batch code		Expiry date
	Caution		Content		Consult instructions for use
	For research use only!				